Structure–Activity Relationships of 33 Piperidines as Toxicants Against Female Adults of *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT *Aedes aegypti* (L.) (Diptera: Culicidae) is the primary vector of both dengue and yellow fever. Use of insecticides is one of the primary ways to control this medically important insect pest. However, few new insecticides have been developed for mosquito control in recent years. As a part of our collaborative effort to search for new insecticides to control mosquitoes, piperidine was used as base compound for further optimization. Herein, we report the structure–activity relationships of 33 piperidines against adult female *Ae. aegypti*. On the basis of 24-h LD_{50} values after topical application, the most toxic compound was 2-ethyl-piperidine, with an LD_{50} as low as 0.8 μg per mosquito. The toxicities of piperidine derivatives were significantly decreased when a benzyl moiety was attached to the carbon of the piperidine ring, with an LD_{50} value as high as 29.2 μg per mosquito. The toxicity order of three moieties attached to the carbon of the piperidine ring was ethyl- > methyl- > benzyl-derivatives. When the same moiety was attached to the piperidine ring, the carbon position to which the moiety was attached conferred different toxicity and the toxicity order was second carbon > third carbon > fourth carbon. Together, these preliminary results may be useful in guiding further piperidine ring modifications in the development of potential new insecticides.

KEY WORDS piperidine, yellow fever, dengue, *Aedes aegypti*, insecticide

Aedes aegypti (L.) (Diptera: Culicidae) transmits viral pathogens of humans, including yellow fever (Gillett and Ross 1955, Philip 1962, Soper 1967, Aitken et al. 1977) and dengue (Mattingly 1967, Rudnick 1967, Coleman and McLean 1973, Degallier et al. 1988), both of which can cause severe human morbidity and mortality. Although there is a safe and effective vaccine for the yellow fever virus, epidemic transmission still occurs in Africa with sporadic cases in South America (Vasconcelos et al. 2001; de Filippis et al. 2002; Valero 2003; Onyango et al. 2004a,b). Dengue is the most important arboviral disease in the world and can cause an undifferentiated fever, dengue fever, dengue hemorrhagic fever, or dengue shock syndrome (Malavige et al. 2004). Annually, dengue epidemics account for several million cases and thousands of deaths worldwide (Teixeira Mda et al. 2005).

Mosquito control in many countries relies primarily on insecticides. After the introduction of synthetic organic insecticides in the 1940s and 1950s, *Ae. aegypti* was eradicated from many areas of the world. The Pan American Health Organization initiated a campaign to use DDT to eradicate *Ae. aegypti* in the Western Hemisphere in the late 1940s (Pinto Severo 1955, Fouque and Carinci 1996). By 1972, *Ae. aegypti* had been eradicated from 73% of the land area and 19 countries (Gubler 1989). However, insecticide resistance developed (Brown and Pal 1971), and the campaign ended in 1972 before the eradication goal was achieved. Insecticide resistance has resulted in significant loss of efficacy to commonly used insecticides. Therefore, there is urgent need for the development of alternative insecticides to control these important disease vectors.

One potential source of new pesticides is natural plant derivatives. Not only might certain natural plant products be a source of new pesticides but also botanical chemical derivatives may be more environmentally friendly than synthetic chemicals. Plants in the family Piperaceae are members of traditional pharmacopeia in many Asian and African cultures, and they also have been used for pest control (Wei and Xu 1998, Srivastava 1970). A petroleum ether extract of *Piper guineese* Schumacher & Thonn. roots showed insecticidal activity against house flies, *Musca domestica* L. (Gbewonyo and Candy 1992). A petroleum ether extract of *Piper chaba* Hunter showed insecticidal activity against the red flour beetle, *Tribolium castaneum* (Herbst) (Wei and Xu 1998). Pipernonaline, a piperidine alkaloid derived from *Piper longum* L., also was found to be active against mosquito larvae

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(Lee 2000, Yang et al. 2002). Piperine [(*E,E*)-1-piperoyl-piperidine] is a major piperidine alkaloid isolated from *Piper nigrum* Linn (Rathnawathie and Buckle 1983). Natural alkaloid piperine and 12 synthetic derivatives have been evaluated against epimastigote and amastigote forms of the protozoan parasite *Trypanosoma cruzi,* the etiological agent of Chagas' disease. Furthermore, it has been suggested that piperine is a suitable template for the development of new drugs with trypanocidal activity (Ribeiro et al. 2004).

As part of our effort to search for new insecticides for mosquito control funded by Deployed War-Fighter Protection program, piperidine was used as a base compound for further optimization as repellents (Katritzky et al. 2006). However, it is not clear whether piperidines also have insecticidal activity. In this study, we evaluated insecticidal activities of 33 derivatives of piperidine against female adults of *Ae. aegypti* and studied the structure–activity relationships of these piperidines. Our results revealed that different moieties attached to the piperidine ring had different toxicities against *Ae. aegypti.* This research will be useful for guiding further modification of the piperidine ring in the development of new and more efficacious insecticides.

Materials and Methods

***Ae. aegypti* Mosquitoes.** The Orlando strain of *Ae. aegypti* was reared in the insectary of the Mosquito and Fly Research Unit at Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), USDA–ARS. This Orlando strain of *Ae. aegypti* has been established in CMAVE since the late 1940s. Female adults were used for all experiments because only females take bloodmeals and are concern of the general public. Eggs were hatched by placing a square of a paper towel with eggs in a flask filled with 1,000 ml of distilled water containing 40 mg of larval diet (3:2 brewer's yeast/liver powder, MP Biomedicals, Irvine, CA). The hatched larvae were held overnight in the flask, and 200 larvae were transferred to a 4-liter plastic tray containing 2 liters of distilled water. Larval diet was added to each tray according to the following schedule: day 1, 80 mg; day 3, 40 mg; day 4, 80 mg; day 5, 120 mg; and day 6, 150 mg. Mosquitoes were reared in an environmental chamber set with a temperature profile representing a simulated summer day regime (ranging from 22 to 30°C) and 80% RH. Incandescent lighting was set to a crepuscular profile with a photoperiod of 14:10 (L:D) h, including 2 h of simulated dawn and 2 h of simulated dusk. Adults were held in a screened cage and provided 10% sucrose ad libitum. Bovine blood in 1% heparin that had been placed in a pig intestine and warmed to 37°C was provided to adults twice a week. Eggs were collected on paper towels (Vasco Brands, Elmira, NY) that lined the rim of water containers. These egg-laden papers were air dried at 27°C and 80% RH for 24 h and stored in containers with 100% humidity for 3–30 d. When needed, eggs were hatched under vacuum and larvae were reared in containers as described above.

Chemicals. All Piperidines were synthesized and the identities were confirmed by mass spectrometry either by the Center for Heterocyclic Compounds (University of Florida, Gainesville, FL) or by the Natural Products Utilization Research Unit (USDA–ARS). Piperine [(*E,E*)-1-piperoyl-piperidine], hydromethylnon, and permethrin were purchased from Chem Service (West Chester, PA).

Adult Bioassays. To determine precisely the toxicity of each piperidine against female *Ae. aegypti,* each chemical was serially diluted in acetone and topically applied to individual mosquitoes. Before insecticide application, 5–7-d-old females were briefly anesthetized for 30 s with carbon dioxide and placed on a 4°C chill table (BioQuip Products, Rancho Dominguez, CA). A droplet of 0.5 μl of insecticide solution was applied to the dorsal thorax using a 700 series syringe and a PB 600 repeating dispenser (Hamilton, Reno, NV). Six concentrations providing a range of 0–100% of mortality were used on 25–30 females per concentration. Tests were replicated three times. Control treatments with 0.5 μl of acetone alone gave control mortality rates of <10%. After treatment, mosquitoes were kept in plastic cups and supplied with 10% sucrose solution for 24 h before mortality was recorded. Temperature and humidity were maintained at 26°C and 80% RH, respectively. Every bioassay was conducted at 27°C and 80% RH and replicated three times. Bioassay data were analyzed using PoloPlus probit and logit analysis software (LeOra Software, Petaluma, CA). Chi-squared goodness-of-fit test was performed and LD_{50}/LD_{95} values were calculated using PoloPlus program.

Results and Discussion

To understand whether the carbon position to which the methyl-moiety was attached would affect the toxicities of piperidines, we evaluated the toxicities of 15 piperidines with methyl-moiety at the second, third, and fourth carbon of the piperidine ring (Fig. 1). As shown in Table 1, the LD_{50} values of 2-methyl-piperidines tested ranged from 1.09 to 1.77 μg per mosquito, with the exception of two saturated long chain derivatives (1-decanoyl- and 1-dodecanoyl), whose LD_{50} values were 2.74 and 8.76 μg, respectively. On average, the toxicities of 3-methyl-piperidines tested were slightly lower than that of 2-methyl-piperidines, with LD_{50} values ranging from 1.80 to 4.14 μg per mosquito. However, there was no significant difference between the toxicities of 3-methyl piperidines and 4-methyl piperidines, whose LD_{50} values ranged from 1.22 to 6.71 μg per mosquito. Again, the toxicities of saturated long chain derivatives of 4-methyl-piperidine were lower than others, with LD_{50} values for 1-decanoyl- and 1-dodecanoyl-piperidines elevated to 4.90 and 6.71 μg per mosquito, respectively.

Next, we evaluated the toxicities of piperidines with two different moieties (ethyl- and benzyl-) attached

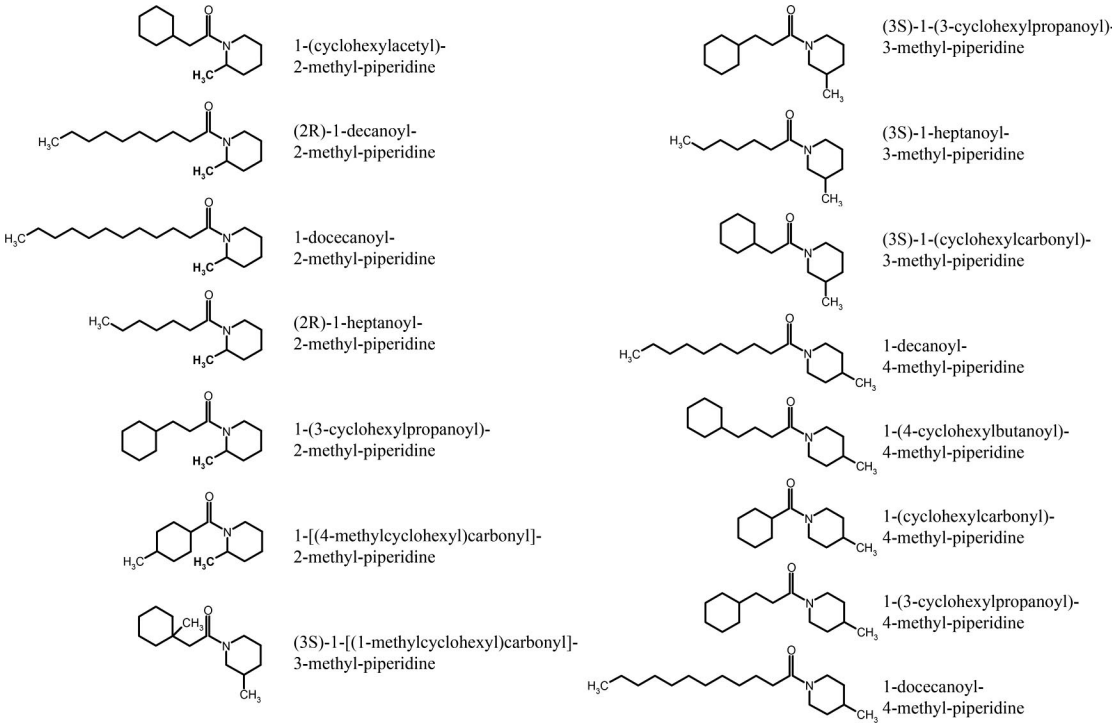

Fig. 1. Chemical structures of the 15 methyl-piperidines listed in Table 1.

to the carbons of the piperidine ring against *Aedes aegypti.* As shown in Table 2 and Fig. 2, the toxicities of 2-ethyl-piperidines were significantly higher than those of benzyl-piperidines. The LD_{50} values of 2-ethyl-piperidines ranged from 0.84 μg to 1.83 μg per mosquito, whereas the LD_{50} values of benzyl-piperidines ranged from 12.89 to 29.20 μg.

Together, the results of Tables 1 and Table 2 suggest that the toxicities of ethyl-piperidines were generally higher than those of methyl-piperidines, followed by benzyl-piperidines whose toxicities were the lowest. However, these piperidines were different compounds with different moieties attached. To validate the finding that moieties directly affected toxicity, we synthesized a series of piperidines with the three moieties (ethyl-, methyl-, and benzyl-) attached to the second, third, and fourth carbon positions of the piperidine ring. As positive control, we included permethrin and hydromethylnon in the bioassays. As shown in Table 3 and Fig. 3, among the three 1-undec-10-enoyl-piperidines with the three different moieties added to the second carbon of the piperidine ring, the 2-ethyl-derivative had the highest toxicity (LD_{50} = 0.80 μg), followed by the 2-methyl-derivative (LD_{50} =

Table 1. Toxicities of 15 analogs of methyl-piperidines to adult female *Ae. aegypti* 24 h after topical application

Chemical	LD_{50} (95% CI)[a]	LD_{95} (95% CI)[a]	Slope (SE)	χ^2
1-(Cyclohexylacetyl)-2-methyl-piperidine	1.77 (1.46–2.27)	4.73 (3.29–10.47)	3.85 (0.79)	0.082
(2*R*)-1-Decanoyl-2-methyl-piperidine	2.74 (1.97–4.34)	26.40 (11.26–283.71)	1.67 (0.41)	0.356
1-Dodecanoyl-2-methyl-piperidine	8.76 (6.62–12.68)	38.86 (22.21–147.56)	2.54 (0.56)	0.111
(2*R*)-1-Heptanoyl-2-methyl-piperidine	1.20 (0.94–1.78)	4.70 (2.68–20.33)	2.77 (0.65)	0.048
1-(3-Cyclohexylpropanoyl)-2-methyl-piperidine	1.09 (0.83–1.68)	5.31 (2.77–34.85)	2.39 (0.61)	0.344
1-[(4-Methylcyclohexyl)carbonyl]-2-methyl-piperidine	1.13 (0.86–1.44)	6.14 (4.07–12.34)	2.23 (0.33)	2.891
(3*S*)-1-(1-Methylcyclohexyl)carbonyl-3-methyl-piperidine	4.14 (3.32–5.88)	14.88 (9.07–43.36)	2.96 (0.58)	0.013
(3*S*)-1-(3-Cyclohexylpropanoyl)-3-methyl-piperidine	1.92 (1.58–2.47)	5.82 (3.87–15.45)	3.41 (0.75)	0.734
(3*S*)-1-Heptanoyl-3-methyl-piperidine	2.07 (1.85–2.38)	4.21 (3.37–6.37)	5.35 (0.89)	0.731
(3*S*)-1-(Cyclohexylcarbonyl)-3-methyl-piperidine	1.80 (1.37–2.75)	10.28 (5.30–48.62)	2.17 (0.46)	0.812
1-Decanoyl-4-methyl-piperidine	4.90 (3.96–5.78)	14.76 (10.97–27.02)	3.44 (0.65)	1.290
1-(4-Cyclohexylbutanoyl)-4-methyl-piperidine	4.25 (3.11–6.00)	23.99 (13.89–69.19)	2.19 (0.39)	0.309
1-(Cyclohexylcarbonyl)-4-methyl-piperidine	2.63 (2.21–3.09)	7.31 (5.46–13.05)	3.71 (0.68)	1.718
1-Dodecanoyl-4-methyl-piperidine	6.71 (5.16–9.14)	31.09 (18.00–123.44)	2.47 (0.57)	0.174
1-(3-Cyclohezylpropanoyl)-4-methyl-piperidine	1.22 (0.94–1.93)	5.34 (2.86–31.00)	2.57 (0.64)	0.053
Piperine [(*E,E*)-1-piperoyl-piperidine]	8.13 (6.10–12.99)	58.74 (28.13–303.44)	1.92 (0.39)	0.539

[a] LD_{50} value in units of micrograms per mosquito.

Table 2. Toxicities of five 2-ethyl- and four benzyl-piperidine analogs to adult female *Ae. aegypti* 24 h after topical application

Chemical	LD_{50} (95% CI)[a]	LD_{95} (95% CI)[a]	Slope (SE)	χ^2
1-(Cyclohexylcarbonyl)-2-ethyl-piperidine	1.67 (1.44–2.08)	4.19 (2.97–8.91)	4.13 (0.84)	1.355
1-(3-cyclohexylpropanoyl)-2-ethyl-piperidine	0.94 (0.70–1.40)	4.28 (2.46–13.03)	2.50 (0.46)	0.241
1-Propionyl-2-ethyl-piperidine	1.56 (1.33–1.78)	3.69 (2.95–5.56)	4.38 (0.72)	0.063
1-(3-Cyclopentylpropanoyl)-2-ethyl-piperidine	1.83 (1.14–2.63)	6.40 (3.90–31.62)	3.03 (0.46)	4.116
1-Nonanoyl-2-ethyl-piperidine	0.84 (0.60–1.10)	5.16 (3.12–14.62)	2.09 (0.40)	0.224
1-Octanoyl-3-benzyl-piperidine	29.20 (19.82–49.09)	371.56 (154.47–2475.31)	1.49 (0.29)	0.343
1-Undec-10-enoyl-4-benzyl-piperidine	14.72 (10.59–25.29)	128.15 (54.95–1114.22)	1.75 (0.40)	0.611
1-Cyclohexylacetyl-4-benzyl-piperidine	19.22 (12.68–42.67)	152.50 (59.76–1778.90)	1.83 (0.43)	0.069
1-(3-Cyclohexylpropanoyl)-4-benzyl-piperidine	12.89 (10.11–17.45)	61.30 (37.01–165.40)	2.43 (0.43)	0.084
Piperine [(*E,E*)-1-piperoyl-piperidine]	8.13 (6.10–12.99)	58.74 (28.13–303.44)	1.92 (0.39)	0.539

[a] LD_{50} value in units of micrograms per mosquito.

1.38 μg). When the benzyl moiety was attached to the same position, the toxicity was significantly decreased (LD_{50} = 3.59 μg). Similarly, among the three 1-undec-10-enoyl-piperidines with the three different moieties attached to the third carbon of the piperidine ring, the 3-ethyl-derivative had the highest toxicity (LD_{50} = 1.32 μg), followed by the 3-methyl- and 3-benzyl derivatives (LD_{50} = 2.07 and 7.43 μg, respectively). Bioassay results of the three 1-undec-10-enoyl-piperidines with the three different moieties attached to the fourth carbon of the piperidine ring revealed the same trend (Table 3; Fig. 3). The 4-ethyl-derivative had the highest toxicity (LD_{50} = 1.54 μg), followed by the 4-methyl-derivative (LD_{50} = 2.72 μg) and the 4-benzyl-derivative (LD_{50} = 14.72 μg). Based on LD_{50} values, we conclude the following: 1) different moieties conferred different toxicities to the piperidines (for example, 2-ethyl- > 2-methyl- > 2-benzyl-; 3-ethyl- >3-methyl >3-benzyl-; 4-ethyl ≥4-methyl >4-benzyl- toxicity), regardless of to which carbon they were attached; 2) when the same moiety was attached to the piperidine ring, the position of the carbon to which the moiety was attached would make a difference and the order of toxicities was second carbon > third carbon > fourth carbon (for example, 2-ethyl > 3-ethyl > 4-ethyl; 2-methyl > 3-methyl- > 4-methyl; 2-benzyl > 3-benzyl > 4-benzyl).

It has been well recognized that natural plant derivatives could be developed into products suitable for pest control because many of them are selective and often biodegrade into nontoxic products (Sukumar et al. 1991, Shaalan et al. 2005). As part of our effort to search for new insecticides for mosquito control, piperidine was used as base compound for further optimization in this study. We observed that the toxicity of the 2-ethyl-1-undec-10-enoyl derivative (LD_{50} = 0.8 μg) was 10 times higher than that of piperine (LD_{50}= 8.13 μg) and almost as toxic as the relatively new insecticide hydromethylnon (LD_{50} =

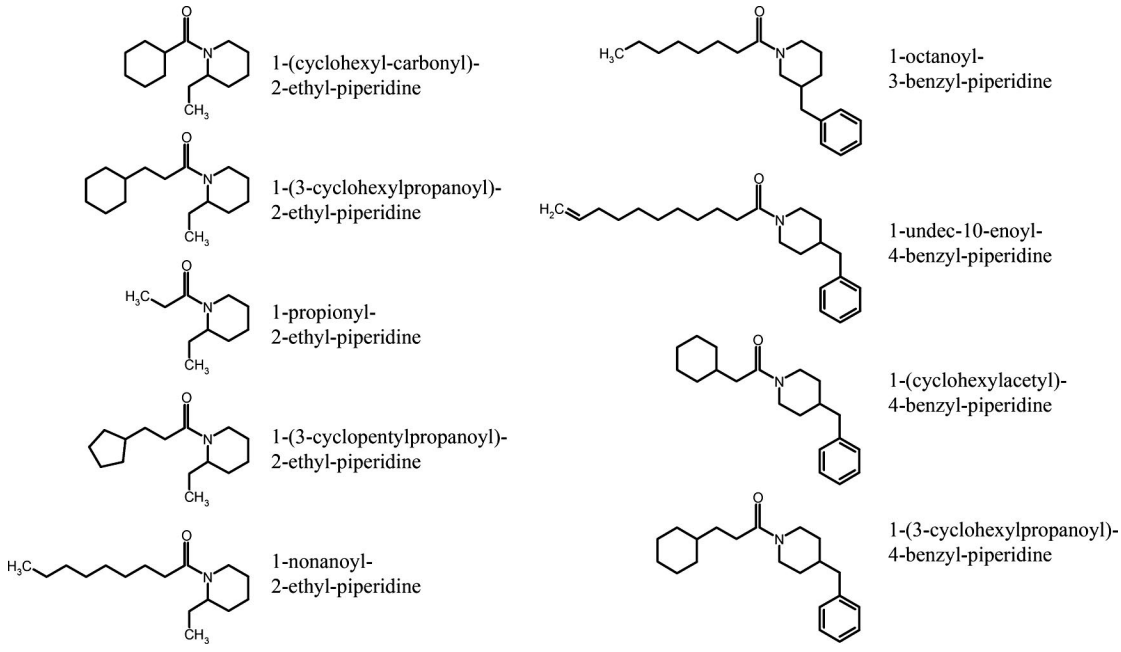

Fig. 2. Chemical structures of the ethyl- and benzyl-piperidines listed in Table 2.

Table 3. Toxicities of nine analogs of 1-undec-10-enoyl-piperidines to adult female *Ae. aegypti* 24 h after topical application

Chemical	LD_{50} (95% CI)[a]	LD_{95} (95% CI)[a]	Slope (SE)	χ^2
2-Ethyl-1-undec-10-enoylpiperidine	0.80 (0.65–0.97)	2.18 (1.60–4.01)	3.75 (0.69)	0.004
2-Methyl-1-undec-10-enoylpiperidine	1.38 (1.12–1.98)	4.55 (2.78–15.30)	3.17 (0.71)	0.219
2-Benzyl-1-undec-10-enoylpiperidine	3.59 (2.68–7.03)	15.49 (7.62–115.56)	2.59 (0.65)	0.131
3-Ethyl-1-undec-10-enoylpiperidine	1.32 (0.97–1.69)	5.38 (3.45–16.13)	2.70 (0.62)	0.103
3-Methyl-1-undec-10-enoylpiperidine	2.07 (1.84–2.33)	4.44 (3.64–6.12)	4.95 (0.68)	1.109
3-Benzyl-1-undec-10-enoylpiperidine	7.43 (6.02–9.68)	25.85 (17.18–55.50)	3.04 (0.51)	1.362
4-Ethyl-1-undec-10-enoylpiperidine	1.54 (1.18–2.71)	5.52 (3.00–30.25)	2.97 (0.74)	0.559
4-Methyl-1-undec-10-enoylpiperidine	2.72 (2.07–3.85)	17.07 (9.09–73.06)	2.01 (0.43)	0.476
4-Benzyl-1-undec-10-enoylpiperidine	14.72 (10.59–25.29)	128.15 (54.95–1114.22)	1.75 (0.40)	0.611
Piperine [(*E,E*)-1-piperoyl-piperidine]	8.13 (6.10–12.99)	58.74 (28.13–303.44)	1.92 (0.39)	0.539
Permethrin	0.00014 (0.00082–0.000246)	0.000343 (0.000208–0.003037)	4.14 (0.61)	2.342
Hydromethylnon	0.57 (0.41–0.74)	2.99 (1.98–6.44)	2.29 (0.40)	1.260

[a] LD_{50} and LD_{95} values are in units of micrograms per mosquito.

0.57 μg) (Table 3), indicating that piperidine might be suitable for further optimization for the development of novel insecticides for mosquito control. Additionally, the toxicities of several ethyl-and methyl-piperidines were significantly higher than that of piperine, indicating that the 1-piperoyl portion of piperine does not play an important role in its toxicity. This result is consistent with Park et al. (2002), in which it was indicated that the 1-piperoyl portion of piperine was not essential for piperine toxicity. Our preliminary data indicated that replacing the 1-piperoyl portion of piperine with different moieties did not reduce its toxicity (unpublished data).

Structure–activity relationships of compounds have been well studied (Elliott et al. 1971; Creemer et al. 2000; Boger et al. 2001; Ito et al. 2002; Park et al. 2002; Wang et al. 2002; Ito et al. 2003a,b; Meegalla et al. 2006). For example, Park et al. (2002) studied the insecticidal activity of different isobutylamides derived from the fruit of *P. nigrum* against third instars of *Culex pipiens pallens* Coquillett, *Ae. aegypti*, and *Aedes togoi* Theobald. Structure–activity relationship studies indicate that the methylenedioxyphenyl moiety of retrofractamide does not play a crucial role in larvicidal activity. Ito et al. (2002, 2003b) synthesized a series of dihydropyrrole derivatives with different moieties added at different positions and studied their insecticidal activities against planthopper *Nilaparvata lugens* Stål and leafhopper *Nephotettix cincticeps* Uhler. Structure–activity relationship studies of these dihydropyrrole derivatives have shown that moieties and positions play important roles in toxicities. Our studies also indicate that different moieties at different positions confer different insecticidal activities (2 > 3 > 4 for position trend; ethyl > methyl > benzyl for moiety trend).

In conclusion, we have evaluated the insecticidal activities of 33 piperidines against female *Ae. aegypti*. Structure–activity relationships indicated that ethyl-was a better moiety suitable as attachment to the piperidine ring to increase the insecticidal toxicity, whereas the benzyl-moiety tended to significantly decrease insecticidal toxicity. Several ethyl-derivatives exhibited ≈10 times higher insecticidal activity than

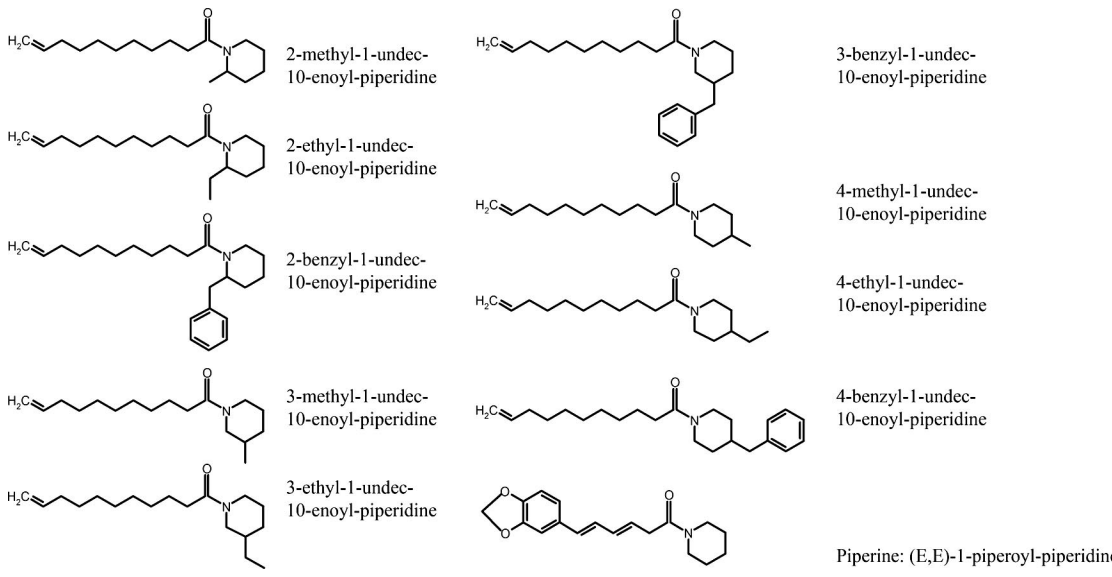

Fig. 3. Chemical structures of piperine and the ethyl-, methyl-, and benzyl-piperidines listed in Table 3.

piperine against female *Ae. aegypti*. These preliminary results may be useful in guiding further modifications of the piperidine ring in the development of potential new insecticides.

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References Cited

Aitken, T. H., W. G. Downs, and R. E. Shope. 1977. *Aedes aegypti* strain fitness for yellow fever virus transmission. Am. J. Trop. Med. Hyg. 26: 985–989.

Boger, M., D. Durr, L. Gsell, R. G. Hall, F. Karrer, O. Kristiansen, P. Maienfisch, A. Pascual, and A. Rindlisbacher. 2001. Synthesis and structure-activity relationships of benzophenone hydrazone derivatives with insecticidal activity. Pest Manag. Sci. 57: 191–202.

Brown, A. W., and R. Pal. 1971. Insecticide resistance in arthropods. Public Health Pap. 38: 1–491.

Coleman, J. C., and D. M. McLean. 1973. Dengue virus transmission by *Aedes aegypti* mosquitoes following intrathoracic inoculation. Am. J. Trop. Med. Hyg. 22: 124–129.

Creemer, L. C., H. A. Kirst, J. W. Paschal, and T. V. Worden. 2000. Synthesis and insecticidal activity of spinosyn analogs functionally altered at the 2′-,3′- and 4′-positions of the rhamnose moiety. J. Antibiot. (Tokyo) 53: 171–178.

de Filippis, A. M., R. M. Nogueira, H. G. Schatzmayr, D. S. Tavares, A. V. Jabor, S. C. Diniz, J. C. Oliveira, E. Moreira, M. P. Miagostovich, E. V. Costa, et al. 2002. Outbreak of jaundice and hemorrhagic fever in the Southeast of Brazil in 2001: detection and molecular characterization of yellow fever virus. J. Med. Virol. 68: 620–627.

Degallier, N., J. P. Herve, A. P. Travassos da Rosa, and G. C. Sa. 1988. *Aedes aegypti* (L.): importance of its bioecology in the transmission of dengue and other arboviruses. I. Bull Soc. Pathol. Exot. Filiales 81: 97–110.

Elliott, M., N. F. Janes, and B. C. Pearson. 1971. The pyrethrins and related compounds. XII. 5-substituted 3-furoates and 3-thenoates, intermediates for synthesis of insecticidal esters. J. Chem. Soc. (Perkin 1) 14: 2551–2557.

Fouque, F., and R. Carinci. 1996. *Aedes aegypti* in French Guiana. Some aspects of history, general ecology and vertical transmission of the dengue virus. Bull. Soc. Pathol. Exot. 89: 115–119.

Gbewonyo, W. S., and D. J. Candy. 1992. Separation of insecticidal components from an extract of the roots of male *Piper guineense* (west African black pepper) by gas chromatography. Toxicon 30: 1037–1042.

Gillett, J. D., and R. W. Ross. 1955. The laboratory transmission of yellow fever by *Aedes (Stegomyia) aegypti* (Linnaeus) from Malaya. Ann. Trop. Med. Parasitol. 49: 63–65.

Gubler, D. J. 1989. *Aedes aegypti* and *Aedes aegypti*-borne disease control in the 1990s: top down or bottom up. Charles Franklin Craig Lecture. Am. J. Trop. Med. Hyg. 40: 571–578.

Ito, M., H. Okui, H. Nakagawa, S. Mio, A. Kinoshita, T. Obayashi, T. Miura, J. Nagai, S. Yokoi, R. Ichinose, et al. 2002. Synthesis and insecticidal activity of N-oxydihydropyrroles: 4-hydroxy-3-mesityl-5,5-dimethyl derivatives with various substituents at the 1-position. Biosci. Biotechnol. Biochem. 66: 2406–2414.

Ito, M., H. Okui, H. Nakagawa, S. Mio, A. Kinoshita, T. Obayashi, T. Miura, J. Nagai, S. Yokoi, R. Ichinose, et al. 2003a. Synthesis and insecticidal activity of novel dihydropyrrole derivatives with N-sulfanyl, sulfinyl, and sulfonyl moieties. Bioorg. Med. Chem. 11: 489–494.

Ito, M., H. Okui, H. Nakagawa, S. Mio, A. Kinoshita, T. Obayashi, T. Miura, J. Nagai, S. Yokoi, R. Ichinose, et al. 2003b. Synthesis and insecticidal activity of novel N-oxydihydropyrroles: 4-hydroxy-3-mesityl-1-methoxymethoxy derivatives with various substituents at the 5-position. Bioorg. Med. Chem. 11: 761–768.

Katritzky, A. R., D. A. Dobchev, I. Tulp, M. Karelson, and D. A. Carlson. 2006. QSAR study of mosquito repellents using Codessa Pro. Bioorg. Med. Chem. Lett. 16: 2306–2311.

Lee, S. E. 2000. Mosquito larvicidal activity of pipernonaline, a piperidine alkaloid derived from long pepper, *Piper longum*. J. Am. Mosq. Control Assoc. 16: 245–247.

Malavige, G. N., S. Fernando, D. J. Fernando, and S. L. Seneviratne. 2004. Dengue viral infections. Postgrad. Med. J. 80: 588–601.

Mattingly, P. F. 1967. *Aedes aegypti* and other mosquitos in relation to the dengue syndrome. Bull. World Health Organ. 36: 533–535.

Meegalla, S. K., D. Doller, R. Liu, D. Sha, Y. Lee, R. M. Soll, N. Wisnewski, G. M. Silver, and D. Dhanoa. 2006. Synthesis and insecticidal activity of fluorinated 2-(2,6-dichloro-4-trifluoromethylphenyl)-2,4,5,6-tetrahydrocyclopentapyrazoles. Bioorg. Med. Chem. Lett. 16: 1702–1706.

Onyango, C. O., A. A. Grobbelaar, G. V. Gibson, R. C. Sang, A. Sow, R. Swaneopel, and F. J. Burt. 2004a. Yellow fever outbreak, southern Sudan, 2003. Emerg. Infect. Dis. 10: 1668–1670.

Onyango, C. O., V. O. Ofula, R. C. Sang, S. L. Konongoi, A. Sow, K. M. De Cock, P. M. Tukei, F. A. Okoth, R. Swanepoel, F. J. Burt, et al. 2004b. Yellow fever outbreak, Imatong, southern Sudan. Emerg. Infect. Dis. 10: 1063–1068.

Park, I. K., S. G. Lee, S. C. Shin, J. D. Park, and Y. J. Ahn. 2002. Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species. J. Agric. Food Chem. 50: 1866–1870.

Philip, C. B. 1962. Transmission of yellow fever virus by aged *Aedes aegypti* and comments on some other mosquitovirus relationships. Am. J. Trop. Med. Hyg. 11: 697–701.

Pinto Severo, O. 1955. [Campaign of eradication of *Aedes aegypti* in the Americas; its organization, development and results since December 1954.]. Bol. Of. Sanit. Panam. 38: 378–398.

Rathnawathie, M., and K. A. Buckle. 1983. Determination of piperine in pepper (*Piper nigrum*) using high-performance liquid chromatography. J. Chromatogr. 264: 316–320.

Ribeiro, T. S., L. Freire-de-Lima, J. O. Previato, L. Mendonca-Previato, N. Heise, and M. E. de Lima. 2004. Toxic effects of natural piperine and its derivatives on epimas-

tigotes and amastigotes of *Trypanosoma cruzi.* Bioorg. Med. Chem. Lett. 14: 3555–3558.

Rudnick, A. 1967. *Aedes aegypti* and haemorrhagic fever. Bull World Health Organ. 36: 528–532.

Shaalan, E. A., D. Canyon, M. W. Younes, H. Abdel-Wahab, and A. H. Mansour. 2005. A review of botanical phytochemicals with mosquitocidal potential. Environ. Int. 31: 1149–1166.

Soper, F. L. 1967. *Aedes aegypti* and yellow fever. Bull. World Health Organ. 36: 521–527.

Srivastava, J. B. 1970. Insecticide & larvicide activity in the extract of *Piper peepuloides* Royle (Piperaceae). Indian J. Exp. Biol. 8: 224–225.

Sukumar, K., M. J. Perich, and L. R. Boobar. 1991. Botanical derivatives in mosquito control: a review. J. Am. Mosq. Control Assoc. 7: 210–237.

Teixeira Mda, G., C. Costa Mda, M. L. Barreto, and E. Mota. 2005. Dengue and dengue hemorrhagic fever epidemics in Brazil: what research is needed based on trends, surveillance, and control experiences? Cad Saude Publica 21: 1307–1315.

Valero, N. 2003. Yellow fever in Venezuela. Invest. Clin. 44: 269–71.

Vasconcelos, P. F., A. P. Rosa, S. G. Rodrigues, E. S. Rosa, H. A. Monteiro, A. C. Cruz, V. L. Barros, M. R. Souza, and J. F. Rosa. 2001. Yellow fever in Para State, Amazon region of Brazil, 1998–1999: entomologic and epidemiologic findings. Emerg. Infect. Dis. 7: 565–569.

Wang, Q., J. Cheng, and R. Huang. 2002. Synthesis and insecticidal evaluation of novel N-(S-amino)sulfenylated derivatives of diacylhydrazines. Pest Manag. Sci. 58: 1250–1253.

Wei, Y., and H. Xu. 1998. Laboratory evaluation of *Piper chaba* against *Tribolium castaneum.* Nongzikeji 2: 11–12.

Yang, Y. C., S. G. Lee, H. K. Lee, M. K. Kim, S. H. Lee, and H. S. Lee. 2002. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. J. Agric. Food Chem. 50: 3765–3767.

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